Formulation and Physicochemical Characterization of Glibenclamide Solid Dispersion Using Various Carriers

ABSTRACT

Aims: Glibenclamide is an oral hypoglycemic agent exhibits inadequate aqueous solubility resulting in poor and unpredictable bioavailability. The study was designed to enhance the solubility and dissolution of glibenclamide by solid dispersion.
Place and Duration of Study: Department of pharmacy, Rajshahi University, Rajshahi, Bangladesh between June 2017 and July 2018.
Methodology Solid dispersions of glibenclamide were prepared by solvent evaporation technique using mixture of PEG-8000, sodium citrate, HPMC as additives in different ratios and subsequently, *in-vitro* dissolution studies were performed. The characterization of solid dispersions was done by Differential Scanning Calorimetry, Powder X-ray Diffractometer, Fourier Transform Infrared Spectroscopy and Scanning Electron Microscope.
Results Out of twelve formulations, the GCHP-4 composed of glibenclamide: HPMC: Na-citrate: PEG-8000 1:1:1:1) demonstrated highest percentage of yield (87.76%) and encapsulation efficiency (95.68%). The maximum dissolution of glibenclamide obtained from GCHP-4 (3.34 µg/ml), which was 5.2-fold higher than that of pure glibenclamide (0.64 µg/ml) at 120 min. The mechanism of increased solubility of glibenclamide from solid dispersion might be resulted due to the conversion of its crystalline form into amorphous state and no interaction between drug and carriers which was confirmed by differential scanning calorimetry and fourier transform infrared spectroscopy respectively.
Conclusion: The dissolution rate of glibenclamide was greatly increased when loaded in solid dispersions which might be responsible for the improvement of its bioavailability in aqueous medium.

***Keywords**:* Glibenclamide; Hydrophilic carrier; Solid dispersion; *In-vitro* Dissolution

1. INTRODUCTION

Oral route is the simplest and easiest way of drug administration. One of the major problems encountered during oral delivery of active principle is poor bioavailability resulting from inadequate aqueous solubility and low absorption of drugs from gastrointestinal tract. About 40 % of newer drugs experienced poor aqueous solubility and bioavailability; and thus, leading to reduction in therapeutic efficacy and sometimes required increment of dose [1]. Glibenclamide (GLB) is an oral antidiabetic agent widely used in the management of type II diabetes mellitus (T2DM) and belongs to Biopharmaceutical Classification System (BCS) Class II drugs [2, 3]. GLB exhibits very poor solubility at 37°C (<0.004 mg/ml) in both acidic and neutral aqueous media. However, at pH > 7, the solubility of the drug slightly increases (0.02 mg/ml) [4]. Thus, poor solubility of GLB may be responsible for its low dissolution rate and unpredictable bioavailability [1,3] . Improvement in the dissolution rate of the poorly soluble drugs is one of the most difficult tasks in modern pharmaceutics.
Several methods were developed to enhance the aqueous solubility and dissolution characteristics of poorly water soluble drugs including solid dispersion, nanotechnology, salt formation, particle size reduction, addition of solvent and/or surface-

active agents [5]. However, solid dispersion is one of the most suitable approaches for improving oral absorption and bioavailability of drugs resulting from the reduction of particle size and thereby, increasing the surface area[4- 8] . Furthermore, dispersion is effective in reducing the agglomeration of particles as well as gives high loading capacity of drugs [9-10] .The use of natural polymers in preparation of solid dispersions for solubility enhancement of poorly water soluble drugs is more beneficial because of their low cost, biocompatibility, and biodegradability [11] .Poloxamer-188 had been previously shown to enhance drug solubilization in phosphate buffer. [12] . The solid dispersion could improve solubility and in-vitro dissolution rate of a poorly water soluble drug by dispersing it at the molecular level in a biologically inert solid carrier.

In this investigation, we attempted the enhancement of solubility of poorly water soluble GLB by solid dispersion technique due to its ease of preparation, productivity and cost effectiveness. Hence, enhancement of aqueous solubility of GLB using an appropriate physical modification is important to maximize its therapeutic activities as well as minimizing toxicity with effective minimal therapeutic dose. Therefore, mixtures of PEG-8000, sodium citrate, HPMC as carriers/additives were used for the formulation of solid dispersions of GLB. The aim of the present study was to enhance the solubility and dissolution rate of GLB by solid dispersion formulation using a simple solvent evaporation technique.

2. MATERIAL AND METHODS

2.1 Materials

GLB was obtained as research sample from Square Pharmaceuticals Ltd. Pabna, Bangladesh. Polyethylene glycol-8000 (PEG-8000), sodium citrate and hydroxy propyl methyl cellulose (HPMC) were procured from Qualikems Fine Chem Pvt. Ltd. (India). The membrane filter (0.20 and 0.45 µm) and methanol were purchased from Toyo Roshi Kaisha Ltd. (Tokyo, Japan) and Merck, Germany respectively. Other chemical and solvents used were of analytical grade.

2.2 Preparation of solid dispersion of glibenclamide

GLB solid dispersions (SD) were prepared by the solvent evaporation method using various carriers/polymers such as PEG-8000, sodium citrate, and hydroxyl propyl methyl cellulose (HPMC). Briefly, 100 mg of GLB was accurately weighed and dissolved in 20 ml of ethanol in which additive and/or carrier were added at different ratios and dispersed under continuous stirring by magnetic stirrer at 200 rpm and thus, allowing sufficient loading of the drug into carriers. The temperature was maintained at 50-60°C to evaporate the solvent from the dispersion system. When materials were about to dry, the magnetic stirrer was stopped and the SD of GLB were further dried at room temperature under vacuum for 24 hours. Finally, dried SD granules passed through 120 mesh screen to get granules of uniform size. Accordingly, twelve SD formulations were prepared and designated as GH-1, GH-2, GH-3, GCH-1, GCH-2, GCH-3, GCHP-1, GCHP-2, GCHP-3, GCHP-4, GCHP-5, GCHP-6 and kept in airtight container (Table 1).

2.3 Determination of the percentage (%) yield

The percentage yield was calculated from the weight of dried SD of GLB (W_1) recovered from each of the batches and the sum of the initial dry weight of starting materials (W_2) using the following equation. [13]

$$\textbf{Yield \%} = \frac{W1}{W2} \times 100$$

Where, W_1= weight of dried SD of GLB, W_2= initial dry weight of materials

2.4 Encapsulation efficiency (%)

Encapsulation efficiency of SDs of GLB was determined by utilizing the modified protocol of Kenneth C. *et al.* as discussed earlier.[14] Approximately, 10 mg of SDs of GLB (equivalent to pure GLB) was weighed accurately and dissolved in 10 ml methanol. The solution was shaken vigorously and filtered, and the filtrate was analysed by spectrophotometer at 228.5 nm for GLB content. The percentage of encapsulation efficiency of SDs of GLB was calculated using the following formula below:

$$\textbf{EE \%} = \frac{\textit{Actual drug content}}{\textit{Theoritical drug content}} \times 100$$

The above procedure was repeated to obtain the encapsulation efficiency as the mean of three replicate measurements.

2.5 Dissolution study

The *in-vitro* dissolution study was carried out with both pure GLB and freshly prepared SD of GLB according to paddle method (USP Apparatus 2) using a dissolution tester (Tianjin Guoming Medicinal Equipment Co., Ltd.).[15-16] Demineralised water was used as dissolution medium. Briefly, sample of GSD (equivalent to 15mg of GLB) was added to 500 ml of water in dissolution vessel. The paddle speed and temperature were maintained at 50±2 rpm and 37.0±0.5˚C, respectively. The dissolution samples (5ml each) were withdrawn at 5, 10, 15, 30, 45, 60, and 120 minutes followed by the replacement of an equal volume of water. The solution was filtered through (Whatmann) filter paper, and the filtrate was transferred to a volumetric flask and diluted with demineralised water up to 25 ml. The absorbance of these solutions was measured by an UV-spectrophotometer (UVmini-1240, Shimadzu) at 228.5 nm using stock solution as a blank. The absorbance of standard GLB was also measured and drug release from all SD of GLB was determined. Three replicates of each dissolution test were carried out and calculated mean values of cumulative drug release were used while constructing the dissolution profile. The absorbance was plotted against concentration and thus concentration of GLB at each point was assayed using a standard curve. The standard solution was prepared by dissolving 37.5 mg GLB in 25 ml methanol and mixed properly. From the solution appropriate quantity of aliquot was transferred to 25 ml volumetric flask and diluted with methanol to get the desired concentration of 1µg/ml, 3µg/ml, 5µg/ml, 10µg/ml and 20µg/ml.

2.6 Solid state characterization

2.6.1 Characterization by DSC (Differential Scanning Calorimetry)

DSC gives a clear idea about the melting and re-crystalline nature of crystalline materials and is useful to understand the crystalline properties of SD of GLB formulation. Thermograms of pure GLB, various carrier and SD of GLB were obtained from DSC (Exstar SII DSC7020, Hitachi High-Tech Science Corporation, Tokyo, Japan). Each sample (3-5 mg) were placed in sealed standard aluminum pans and heated from 0 to 296˚C, at a scanning rate of 10˚C/min under nitrogen purge with an empty aluminum pans as reference. [17]

2.6.2 Characterization by PXRD (Powder X-ray Diffraction)

An X-ray diffractometer (RAD-C, Rigaka Denki Co. Ltd. Tokyo, Japan) was used for the diffraction studies. The samples were exposed to Cu-Kα radiation (30 kV and 50 mA) and scanned from 3-40˚C, 2θ at a scanning rate of 5˚C/min. [17]

2.6.3 Characterization by FTIR (Fourier-transform infrared spectroscopy)

The nature of drug-carriers interactions in the SD of GLB was analysed using IR spectra measured by the diffuse reflection method using an FTIR spectrometer (IR-Prestige 21, Shimadzu Co. Japan). Disk of samples were prepared by grounding and thorough mixing with potassium bromide. The scanning range was 400 to 4000 cm^{-1} and the resolution was 1cm^{-1}. [17]

2.6.4 Characterization by SEM (scanning electron microscope)

The shape, surface and cross-sectional morphology of pure GLB and SD of GLB were observed using a scanning electron microscope (SSX-500, Shimadzu, Japan) after platinum metallization. An accelerating voltage of 15 kV was used. [17]

2.7 Statistical analysis

Data are expressed as mean ± SEM (standard error of mean). Student's t-test was performed to compare between two groups. One way analysis of variance (ANOVA) was used to assess differences among groups. A value of $p<0.001$ was considered as statistically significant. All statistical analysis was performed by using GraphPad Prism version 20.

3. RESULTS AND DISCUSSION

3.1 RESULTS

Table 1 shows the percentage of yield (% yield) and encapsulation efficiency (% EE) of different GSD formulations. The percentage of yield obtained from GSD ranges from 75.43% to 87.76%. The maximum yield was obtained from GCHP-4 (87.76%) indicating that the manufacturing process might be reliable and can be adopted for future work. [13] Further, the highest encapsulation efficiency was demonstrated by GCHP-4 (95.68%) and might be due to the maximum adsorption of GLB on to the surface of additives.

Table 1. Composition, percentage of yield and encapsulation efficiency of different GLB formulations

Formulation code	Drug: additive/carrier	Weight ratio	% Yield	%EE
GH-1	GLB: HPMC	1:1	79.83	82.3±0.84
GH-2	GLB: HPMC	1:2	80.28	83.45±1.2
GH-3	GLB: HPMC	1:3	73.88	81.94±0.96
GCH-1	GLB: Na citrate: HPMC	1:1:1	85.71	84.53±0.28
GCH-2	GLB: Na citrate: HPMC	1:2:1	80.93	85.06±0.77
GCH-3	GLB: Na citrate: HPMC	1:3:1	78.91	75.93±0.98
GCHP-1	GLB: Na citrate:HPMC:PEG-8000	1:1:2:1	80.91	87.81±0.86
GCHP-2	GLB: Na citrate:HPMC:PEG-8000	1:1:2:2	77.99	85.87±0.69
GCHP-3	GLB: Na citrate:HPMC:PEG-8000	1:1:2:3	75.43	85.87±0.79
GCHP-4	GLB: Na citrate:HPMC:PEG-8000	1:1:1:1	87.76	95.68±0.97
GCHP-5	GLB: Na citrate:HPMC:PEG-8000	1:1:2:1	83.55	90.65±0.95
GCHP-6	GLB: Na citrate:HPMC:PEG-8000	1:1:3:1	81.57	91.69±0.56

HPMC, hydroxyl propyl methyl cellulose; PEG-8000, poly ethylene glycol; Na-citrate, sodium citrate; EE, Encapsulation Efficiency

3.1.1 Dissolution study

In-vitro dissolution study was performed to optimize the ratio of single or multiple additives and/or carriers used to prepare GLB formulations; and compared with that of pure GLB.

Table 2. Dissolution of GSD formulation and standard GLB at different time intervals

Formulation	Drug dissolved (µg/ml)						
	5 min	10 min	15 min	30 min	60 min	90 min	120 min
GLB	0.54±0.01	0.55±0.02	0.58±0.02	0.59±0.01	0.60±0.01	0.62±0.02	0.64±0.02
GH-1	0.94±0.02‡*	0.97±0.01‡*	0.98±0.01‡*	1.01±0.01‡*	1.04±0.01‡*	1.07±0.02‡*	1.02±0.02‡*
GH-2	1.06±0.02‡*	1.13±0.01‡*	1.16±0.01‡*	1.18±0.01‡*	1.21±0.01‡*	1.29±0.03‡*	1.32±0.07‡
GH-3	0.86±0.02‡*	0.93±0.02‡*	0.94± 0.03†*	0.99±0.01‡*	1.00±0.01‡*	1.02±0.01‡*	1.01±0.02‡*
GCH-1	1.16 ±0.02‡*	1.18±0.01‡*	1.23±0.01‡*	1.27±0.01‡*	1.30±0.01‡*	1.38±0.02‡*	1.63±0.02‡*
GCH-2	1.14± 0.02‡*	1.19± 0.01‡*	1.18± 0.01‡*	1.25±0.01‡*	1.29±0.01‡*	1.40±0.03‡*	1.62±.02‡*
GCH-3	0.96±0.02‡*	1.01±0.01‡*	1.03± 0.01‡*	1.05±0.01‡*	1.08±0.01‡*	1.17±0.01‡*	1.22±0.02‡*
GCHP-1	1.98±0.02‡	2.04±0.02‡	2.11±0.01‡	2.13±0.01‡	2.18±0.01‡	2.21±0.01‡	2.27±0.02‡
GCHP-2	1.30±0.02‡*	1.31±0.02‡*	1.35±0.01‡*	1.40±0.01‡*	1.40±0.01‡*	1.41±0.01‡*	1.42±0.01‡*

GCHP-3	1.31±0.01‡*	1.34±0.01‡*	1.36±0.01‡*	1.40±0.01‡*	1.43±0.01‡*	1.43±0.01‡*	1.47±0.01‡*
GCHP-4	2.47±0.01‡*	2.58±0.02‡*	2.64±0.01‡*	2.78±0.01‡*	2.96±0.01‡*	3.19±0.01‡*	3.34±0.01‡*
GCHP-5	1.36±0.01‡*	1.36±0.02‡*	1.38±0.02‡*	1.39±0.01‡*	1.45±0.01‡*	1.50±0.01‡*	1.59±0.01‡*
GCHP-6	1.32±0.01‡*	1.33±0.02‡*	1.35±0.01‡*	1.37±0.01‡*	1.40±0.01‡*	1.44±0.01‡*	1.48±0.01‡*

Data expressed in mean ± SEM, †p<0.001, ‡p<0.0001 vs GLB, and *p<0.0001 vs GCHP-1, Number/frequency of each experiment was 3

3.1.1.1 Optimization of in-vitro drug dissolution using HPMC

The dissolution profiles of newly prepared GLB formulations are shown in Table 2. Primarily, SDs formulations were prepared by adding GLB with a hydrophilic polymer HPMC at different ratios of 1:1, 1:2 and 1:3 (GLB: HPMC); namely GH-1, GH-2, GH-3 respectively. Dissolution profiles of GLB formulations were compared with pure GLB at each sampling point (5, 10, 15, 30, 45, 60 and 120 min). The results demonstrated that at initial sampling time (5 min), the *in-vitro* drug dissolution concentration of GLB from GH-1, GH-2 and GH-3 were 1.74, 1.96 and 1.59-fold respectively higher than that of pure GLB (0.54 µg/ml). Likewise, at 30 min, the dissolution of GLB from GH-1, GH-2, and GH-3 were 1.71, 2.0 and 1.68-fold respectively higher than that of pure GLB (0.59µg/ml). At 120 min, the maximum dissolution of GLB from GH-1, GH-2, and GH-3 were 1.59, 2.06 and 1.58-fold respectively higher when compared to pure GLB (0.64 µg/ml) as shown in Figure1a. Thus, the drug released from GH-1, GH-2 and GH-3 was significantly greater that of pure GLB at each sampling point ($p<0.0001$). Among them GH-2 was found to enhance higher drug release when compared with GH-1 and GH-3; and therefore, may be considered as the optimum formulation with maximum dissolution of GLB [18].

3.1.1.2 Optimization of in-vitro drug dissolution from GH-2 using Na-citrate

Different ratios of Na-citrate along with GH-2 were used to formulate new GSD formulations consisting of GLB, HPMC and Na-citrate at the ratio of 1:2:1, 1:2:2 and 1:2:3, respectively, designated as GCH1, GCH-2, GCH-3 and their dissolution profiles were compared with GLB as shown in Table 2. Here, the data indicated that at 5 min, the dissolution concentration of GLB from GCH-1, GCH-2, and GCH-3 were 2.15, 2.11 and 1.78-fold respectively, at 30 min 2.15, 2.11 and 1.78-fold respectively and at 120 min 2.55, 2.53 and 1.91-fold respectively higher, when compared with pure GLB as shown in Figure1b. Furthermore, the enhanced drug concentrations produced by SDs of GLB were significantly higher than that of pure GLB at each sampling point ($p<0.001$). Among these GCH-1 was found to exhibit highest drug dissolution than that of GCH-2 and GCH-3. GCH-1 was found to enhance drug dissolution significantly in comparison to GCH-3 but no significant difference between GCH-2 and GCH-1 were observed.

3.1.1.3 Optimization of in-vitro drug dissolution from GCH-2 using PEG-8000

The drug dissolution was further optimized by adding a hydrophilic polymer (PEG-8000) in GCH-1 formulation (GLB: HPMC: Na-citrate: PEG-8000) at different ratios of 1:2:1:1, 1:2:1:2 and 1:2:1:3, designated as GCHP-1, GCHP-2 and GCHP-3, respectively and the dissolution profiles were compared with GLB (Table 2). The data demonstrated that at 5 min, dissolution of GLB from GCHP-1, GCHP-2, and GCHP-3 were 3.67, 2.41 and 2.43-fold respectively, at 30 min were 3.61, 2.37 and 2.37-fold respectively and at 120 min, the corresponding increment of drug dissolution was 3.55, 2.22 and 2.30-fold respectively higher when compared with pure GLB as shown in Figure1c. After optimization, the drug release from GCPH-1, GCPH-2 and GCPH-3 were highly significant in comparison to that of pure GLB at each sampling point ($p<0.0001$). Among them, GCHP-1 was found to exhibit enhanced drug dissolution profile in comparison to GCHP-2, GCHP-3 and GLB.

1(a)

1(b)

1(c)

1(d)

Figure 1: (a) Dissolution of GLB from GH-1, GH-2, and GH-3; (b) Dissolution of GLB from GCH-1, GCH-2 and GCH-3; (c) Dissolution of GLB from GCHP-1, GCHP-2, and GCHP-3; (d) Dissolution of GLB from GCHP-4, GCHP-5 and GCHP-6.

3.1.1.4 Optimization of in-vitro drug dissolution using both HPMC and PEG-8000 in GCHP-1

GCHP-1 significantly elevated dissolution of GLB by 3.67-fold higher (at 5 min) than that of pure GLB. Previously, HPMC alone optimized the solubility increment by 1.96-fold, and both HPMC and Na-citrate were by 2.15-fold higher that than of

pure GLB. The fast drug release showed by GCHP-1 might be due to the combined use of two hydrophilic polymers (HPMC and PEG-8000) in the formulation. Therefore, this synergistic effect of these polymers can be optimized by changing their ratios. For this reason, three new formulations namely GCHP-4, GCHP-5 and GCHP-6 composed of GLB: HPMC: Na-citrate: PEG-8000 at the ratio of 1:1:1:1, 1:2:1:1 and 1:2:1:1, respectively were formulated; where the concentration of PEG-8000 kept constant while the concentrations of HPMC varied and the dissolution profile of newly developed GLB formulations is presented in Table 2. Data revealed that at 5 min, the dissolution of GLB from GCHP-4, GCHP-5, and GCHP-6 were 4.57, 2.52 and 2.44-fold respectively, at 30 min were 4.71, 2.36 and 2.32-fold respectively and at 120 min 5.22, 2.48 and 2.31-fold respectively than that of pure GLB as shown in Figure1d. Therefore, the drug dissolution from GCPH-4, GCPH-5 and GCPH-6 were significantly higher in comparison to that of pure GLB at each sampling point ($p<0.0001$) and GCHP-4 was found to exhibit maximum drug release amongst them.

3.1.2 Solid state characterization of GSD

3.1.2.1 Thermal analysis by DSC

The thermal stability of the GLB formulations was assessed and respective DSC thermograms are shown in Figure 2. In the thermograms, sharp endothermic peaks for GLB, PEG-8000 and Na-citrate was found at 177.6˚, 60.1˚ and 162.5˚C, respectively, demonstrating the crystalline property of those materials. A broad peak at 93.5˚C was observed by HPMC indicating poor crystalline behaviour of the additive. The thermogram of GCHP-4 showed two endothermic peaks of which one broad peak at 89.8˚C was corresponding to that of HPMC and another sharp at 50.9˚C corresponding to PEG-8000; probably due to the deformation by the later one. However, the thermogram of GCHP-4 exhibited no peak corresponding to GLB indicating the conversion of crystals to amorphous state.

3.1.2.2. Characterization of crystallinity by PXRD

The X-ray diffraction patterns of pure GLB, HPMC, PEG-8000, Na-citrate and GCHP-4 are shown in Figure 2. The diffraction spectrum of pure GLB exhibited numerous distinctive sharp peaks with high intensity at 2θ angles 11.57˚, 12.28˚, 12.82˚, 14.53˚, 15˚, 16.2˚, 18.51˚, 18.55˚, 19.81˚, 21.64˚, 25.62˚ and 30.1˚ indicating highly crystalline nature of the drug. [19] The extent of crytallinity affects the dissolution of the drug. The carrier PEG-8000 also showed two sharp peaks for crystallinity at 19.35˚ and 23.53˚. The dissolution enhancer Na-citrate also showed four sharp peaks for crystallinity at 27.55˚, 30.27˚, 32.03˚ and 37.3˚. But the carrier HPMC showed no sharp peak indicating its amorphous state. However, no peaks with significant intensity corresponding to GLB were displayed in GCHP-4 demonstrating some possible interaction during mixing process. In the formulation, the peaks corresponding to PEG-8000 appeared as amorphous in nature representing its basic shape. This might be due to some physicochemical interactions among the drug and other additives and need to be confirmed by FTIR study later on. [20]

Figure 2. DSC thermograms of (a) GLB, (b) HPMC, (c) PEG-8000, (d) Na-citrate and (e) GCHP-4.

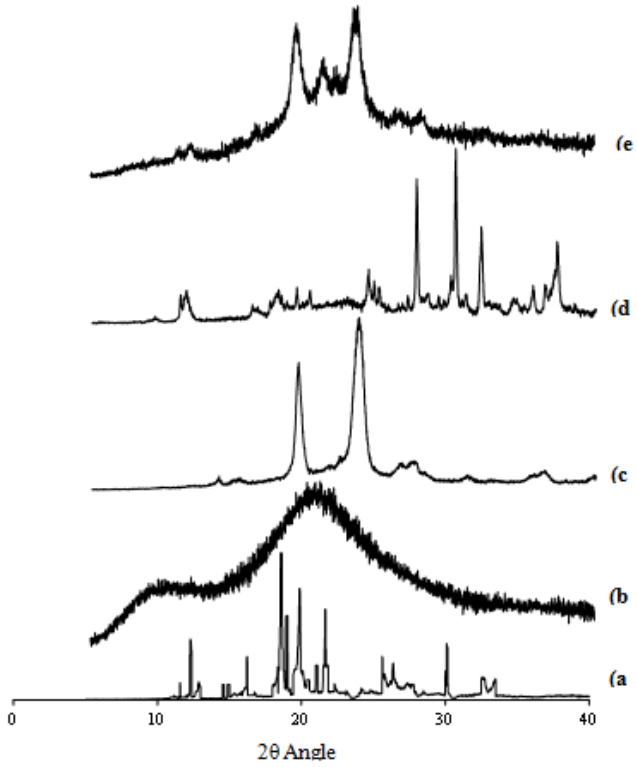

Figure 3. Powder X-ray diffraction patterns of (a) GLB, (b) HPMC, (c) PEG-8000, (d) Na-citrate; and (e) GCHP-4.

3.1.2.3 Interaction study by FT-IR

The FT-IR spectra of GLB, HPMC, PEG-8000, Na-citrate, and the formulation are shown in Figure 4. FT-IR spectroscopy was performed to characterize the possible interactions among the drug and carriers used in the optimized formulation, GCHP-4. In IR spectra GLB showed major distinguished peaks at 1616, 1713, 3312, 1157 and 1341, and 1094 cm^{-1}due to carbonyl group (=CO) of urea, carbonyl group of amide, N-H stretching of amide, sulphonyl group (O=S=O) stretching, N-H bending of urea group, respectively. [21] HPMC generated bands at 2927 and 1060 cm^{-1} for C-H stretching and aliphatic C-O stretching, respectively. Similarly, PEG-8000 showed bands at 2893, 1467 and 1340, 1279 and 1094 cm^{-1} for C-H stretching, C-H bending, C-O-H stretching and O-H stretching respectively. [22] Accordingly, Na-citrate generated bands at 3442, 1585 and 1387 due to O-H stretching, COO^- oscillation and deformation mode of $–CH_2$ stretching respectively. [23-24]

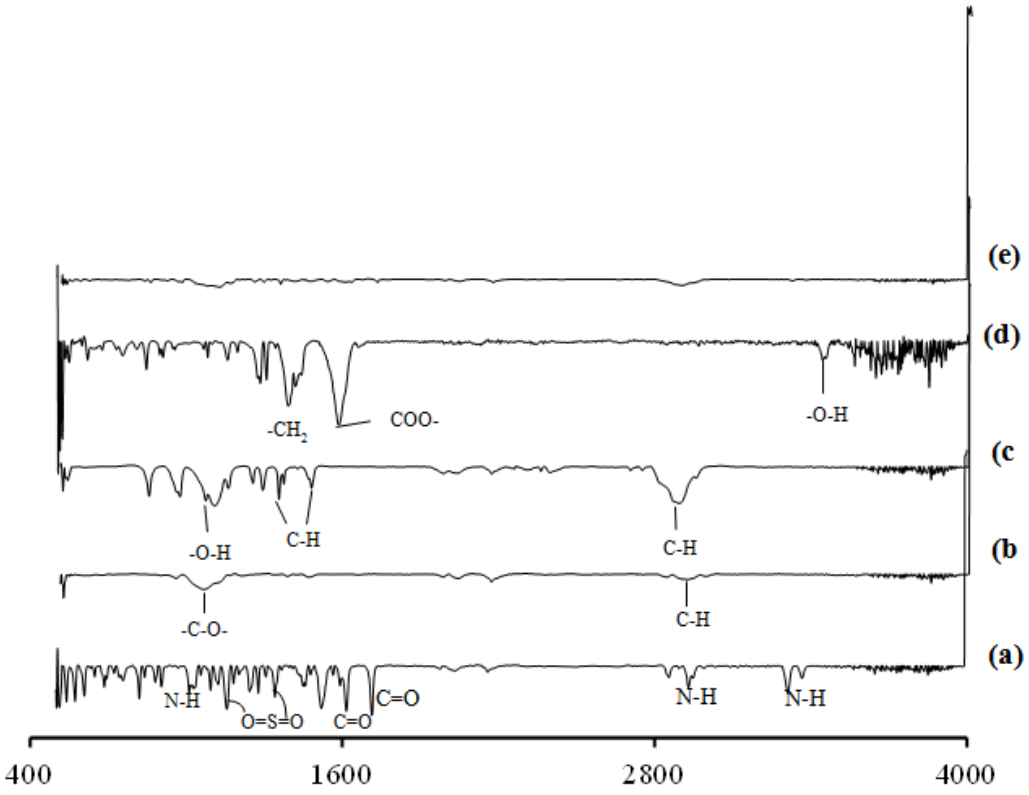

Figure 4 FT-IR spectra of (a) GLB, (b) HPMC, (c) PEG-8000, (d) Na-citrate and (e) GCHP-4

In the spectra of GCHP-4, the intensity of band due to stretching of –NH (at 2931, 3312 cm^{-1}) of urea and amide was disappeared. This might be due to the possible interaction (N-H bridging) between amide (N-H) group of GLB with C-H of PEG (at 2893 cm^{-1}) and HPMC (at 2927 cm^{-1}). The dissolution enhancement might be occurred due to formation of O-H bridging between N-H (at 1094 cm^{-1}) of GLB and with –OH (at 1094 cm^{-1}) of PEG-8000. The intensity of band for N-H bending (1094 cm-1) was disappeared due to formation of O-H bridging between N-H group (at 1094 cm^{-1}) of GLB and O-H group (at 1094 cm-1) of PEG-8000. The intensity of band for stretching of carbonyl group (at 1713 and 1616 cm^{-1}) were absent in FT-IR spectra of GCHP-4. This might be due to the possible interaction between carbonyl group (at 1616 cm^{-1}) of GLB with COO^- (at 1585 cm^{-1}) of Na-citrate. The intensity of band for stretching of sulphonyl group (at 1341 & 1157 cm^{-1}) was abolished in FT-IR spectra of GCHP-4 possibly due to the interaction between sulphonyl group (at 1341 and 1157 cm^{-1}) of GLB with -C-O-H (at 1279 cm^{-1}) of PEG-8000. Thus, FT-IR spectra of GCHP-4 revealed that there were several possible interactions occurred among the additives and GLB present in the formulation, which would be further confirmed by NMR study.

3.1.2.4 Morphological study by SEM

As shown in Figure 5, the microphotograph of pure GLB displayed as irregular crystalline solids; and SEM images HPMC, PEG-8000 and Na-citrate exhibited as amorphous, cubic crystal and agglomerated amorphous form, respectively. However, in GCHP-4, amorphous form of Na-citrate was disappeared that further indicating that an interaction between GLB and Na-citrate might have occurred. The crystalline property of GLB was deformed onto the surface of HPMC, which was previously interpreted by FT-IR as a strong interaction between N-H group of GLB and C-H group of HPMC. Furthermore, the interaction between N-H group of GLB and O-H group of PEG was evident by the SEM image of GCHP-4, in which the conjugate product of GLB and PEG was chemisorbed onto the surface of HPMC also. These might be a possible reason for existence of the formulation in amorphous form; and thus enhancing the dissolution property of GCHP-4.

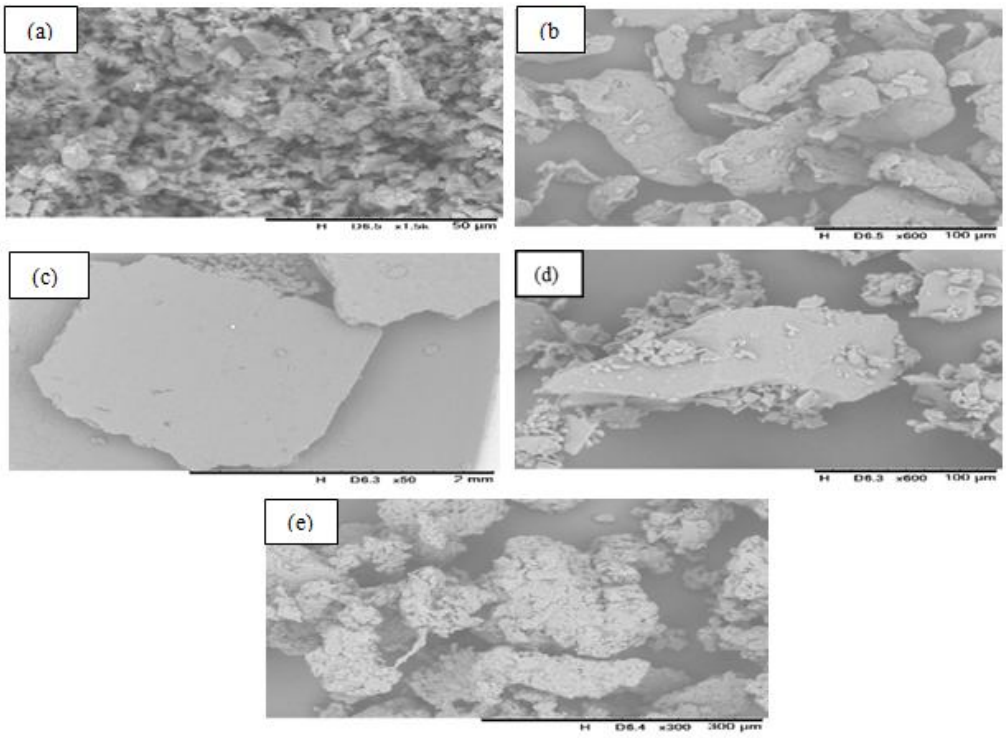

Figure 5. SEM images of (a) GLB, (b) HPMC, (c) PEG-8000, (d) Na-citrate and (e) GCHP-4.

3.2 DISCUSSION

GLB is belongs BCS class II drug which is practically insoluble in water and thus, low oral absorption from gastrointestinal tract [25, 26]. The absorption of GLB was limited by its dissolution rate [27-34]. However, among several methods, solid dispersion technique has been widely used to augment dissolution rate of GLB [28, 32-34]. Many types of carriers such as poloxamer, superdisintegrants, PEG-4000, PEG-8000 and cellulose derivatives were used to prepare solid dispersion. Here, we have attempted a novel approach to improve the solubility of GLB using combination of various additives and dissolution enhancer. Therefore, the rationale of the present study was to investigate the combined effect of PEG-8000, sodium citrate, HPMC as additive on the solubility and dissolution profile of solid dispersion of GLB prepared by solvent evaporation method. In the present investigation twelve formulations were prepared by solvent evaporation method of which GCPH-4 (GLB: HPMC: Na-citrate: PEG-8000, 1:1:1:1) formulation was considered as the most effective. Among different approaches solid dispersions systems have been considered as one of the easiest and less expensive means of increasing the solubility of poorly water soluble drugs [35]. When the solid dispersion is exposed to aqueous media, the carrier dissolves and the GLB released as fine colloidal particles resulting in enhanced surface area, and thus created higher dissolution rate and bioavailability of GLB.

In fact, the optimized GCPH-4 showed maximum percentage of yield (87.76%) and encapsulation efficiency (95.68%) indicating its high production and entrapment capacity. It has been reported that solid dispersion methods have excellent encapsulation efficiency unlike liposomes [14]. It has been previously reported that glibenclamide solid dispersion exhibited 3-fold higher release of drugs than that observed with pure drug [11,36] .One relevant research was carried out by Sharma J

et al who reported that glibenclamide solid dispersion exhibited a maximum release of glibenclamide after 2hrs when exposed to7.4 pH phosphate buffer and which was 3-fold higher than that observed with pure drug [11].

Another study was performed by Tabbakhian M, which showed that glibenclamide dissolution profiles of up to 2 h while exposing to phosphate buffer (pH 9.5) indicated dissolution of drug prepared by the solvent evaporation technique ranged from 61-99 % and only 28% from pure GLB which exhibits 3.54 times higher dissolution in glibenclamide solid dispersion than that of plain glibenclamide[36] . In that experiment, combination of HPMC, PEG-6000 and Poloxamer were used in various fractions. In this study, the maximum dissolution of the drug was obtained from formulation GCHP-4 (3.34 µg/ml) which was 5.2-fold higher than that of pure GLB (0.64 µg/ml) at 120 min. This higher dissolution of GLB primarily attributed to improved hydrophilicity of GLB after the preparation of GCPH-4 which in turn might be occurred due to the chemisorption on HPMC and increased wettability of PEG-4000 that was confirmed by physicochemical characterization. At the initial step, maximum drug dissolution was obtained from the formulation GH-2 (1.32µg/ml at 120min) where GLB was mixed with twice amount of HPMC (GLB: HPMC, 1:2). However, results demonstrated that GCPH-4 showed greater drug dissolution than that of GH-2 where an additional hydrophilic polymer PEG-8000 was used along with HPMC but at 1:1 ratio (HPMC; PEG-8000, 1:1) indicating that these two hydrophilic polymers might have synergistic effects on drug dissolution, and that would be further characterized by DSC, PXRD, FTIR and SEM analysis.
DSC thermograms of GCPH-4 formulations showed that the GLB was converted into amorphous form from its crystalline nature [37] which was, demonstrated by PXRD data also. Previously, it was reported that the conversion of crystalline to amorphous is the major cause of enhanced solubility of drugs when prepared as solid dispersions [38]. No new peak was apparent in DSC thermograms of GCHP-4, suggesting that the crystalline materials used in formulation were completely converted to amorphous form. It was also suggested that GLB is chemically compatible with HPMC, PEG-8000 and Na-citrate [38]. The other mechanism, such as physical bonding between the additives and GLB were demonstrated by FT-IR spectrum. As no peaks with significant intensity corresponding to GLB were found in GCHP-4 indicating some possibility of interaction during mixing process which could be responsible for improving the wettability of GLB and further increasing its dissolution rate [39-41]. SEM photographs showed the particle size and surface morphology of GCHP-4 and hence authenticated that the drug GLB was converted into amorphous form and might be contributing to the enhanced dissolution rate of GLB. Taken together the results of DSC, PXRD, FT-IR and SEM studies, the crystalline behaviour of GLB was completely altered into amorphous one using the carriers (HPMC, PEG and Na-citrate) and thus, the dissolution of GLB in aqueous medium has been extensively increased. The possible mechanism for the enhanced dissolution of GLB from GCHP-4 resulted due to the increased wettability of the GLB by the carrier, drug particle size reduction, polymorphic transformation of drug crystals and chemical interactions between drug and carrier after solid dispersion preparation [42].

4. Conclusion

In the present investigation solid dispersion of GLB was prepared by solvent evaporation method and among the twelve formulations GCHP-4 provided best dissolution profile in comparison with practically insoluble pure GLB. The formulation GCHP-4 was considered the most efficient formulation in terms of its physicochemical properties and dissolution rate. The optimized GCHP-4 showed lowest degree of crystallinity and interaction with additives; and also demonstrated that the drug GLB was converted into an amorphous when formulated with HPMC, Na-citrate and PEG-8000. The GCPH-4 would be beneficial in reducing dose and improving patient compliance of GLB as a result of its increased solubility, dissolution rate and ultimately, would improve absorption from gastrointestinal tract (GIT). However, further studies are required to elucidate the pharmacokinetic characteristics of the optimized formulation of GLB in diabetic patients.

REFERENCES

1.Krishnamoorthy V *et al*. Characterization of olanzapine-solid dispersions. *Iran J Pharm Res* 2011; 10(1):13-24.

2.Coppack SW *et al*. Pharmacokinetic and Pharmacodynamic studies of Glibenclamide in non-insulin diabetes mellitus. *Br. J. Clin. Pharmac.* 1990; 29: 673-684.

3.Amidon GL *et al*. Theoretical basis for a biopharmaceutical drug classification: the correlation of *in vitro* drug product dissolution and in vivo bioavailability. *Pharm Res* 1995; 12(3):413-420.

4. The United States Pharmacopoeia/The National Formulary (USP XXIII/ NF XVIII).

5.Schmid D *et al*. Glibenclamide reduces proinflammatory cytokines in an *ex vivo* model of human endotoxinaemia under hypoxaemic conditions. *Life Sciences* 2011; 89(19– 20): 725–734.

6.Groop LC. Sulfonylureas in NIDDM. *Diabetes Care* 1992; 15(6): 737-54.

7. Abdallah DM *et al*. Glibenclamide ameliorates ischemia-reperfusion injury via modulating oxidative stress and inflammatory mediators in the rat hippocampus. *Brain Research* 2011; 1385: 257–262.

8. Karolewicz B *et al.* Solid dispersion in pharmaceutical technology. Part I. Classification and methods to obtain solid dispersions. *Polim Med* 2012; 42(1): 17-27.

9.Rasenack N *et al*. Microcrystals for dissolution rate enhancement of poorly water-soluble drugs. *Int J Pharm* 2003; 254:137-45.

10.Moyano JR *et al*. Solid-state characterization and dissolution characteristics of gliclazide-betacyclodextrin inclusion complexes. *Int J Pharm* 1997; 148:211-7.

11. Jyoti Sharma, Manju Nagpal, Sandeep Arora. Glibenclamide solubility enhancement by modified natural carriers using the solid dispersion technique. Farmacia, 2012, 60(6):00-00.

12. Sola D *et al*. Sulfonylureas and their use in clinical practice. *Arch Med Sci*. 2015; 11(4):840-8.

13.Sevgi F *et al*. Studies on mefanamic acid microparticles; formulations, *in vitro* release and in situ studies in rats. *AAPS Pharm Sci Tech* 2009; 10(1):104-112.

14.ArunPrasad K *et al*. Preparation and evaluation of solid dispersion of terbinafine hydrochloride. Int J Pharm Sci Rev Res 2010; 3(1):130-134.

15.Mauger J *et al*. Intrinsic Dissolution Performance Testing of the USP Dissolution Apparatus 2 (Rotating Paddle) Using Modified Salicylic Acid Calibrator Tablets: Proof of Principle. *Dissolut Technol* 2003; 10(3):6-15

16.Matkar RD *et al*. USP/IP/NON USP Dissolution Apparatus. Int. *J. Pharm. Life Sci* 2012; 2(4): 40-67.

17. Barman RK et al. Development of Highly Stable Nifedipine Solid-Lipid Nanoparticles. Chem. Pharm. Bull 2014; 62(5): 399-406.

18. Dhillon B *et al*. Formulation and Evaluation of Glibenclamide Solid Dispersion Using different Methods. *Int. J. Pharmacol* 2014; 8 (4): 551-556.

19.Upadhyay P, Pandit J K. Formulation of Fast-Release Gastroretentive Solid Dispersion of Glibenclamide with Gelucire 50/13. *TROP J PHARM RES* 2012; 11 (3): 361-369.

20.Singh K *et al*. Fast dissolving tablet: A novel approach for delivery of Glibenclamide. Res. rev. j. pharm. nanotechnol 2013; 1(1).

21.Thongnopkoon T, Puttipipatkhachorn S. New metastable form of glibenclamide prepared by redispersion from ternary solid dispersions containing polyvinylpyrrolidone-K30 and sodium lauryl sulphate. *Drug Development and Industrial Pharmacy* 2017; 42:1, 70-79.

22.Shameli *K et al*. Synthesis and characterization of polyethylene Glycol Mediated Silver Nanoparticles by the Green Method. *Int. J. Mol. Sci* 2012; 13(6): 6639-6650

23.Lakshmanan BR *et al*., Ibid 1965.38.

24.West *et al*. Chemical Applications of Spectroscopy. *Interscience Publishers Inc.,* New York, 1956.

25.Dastmalchi S *et al.* Enhancing dissolution, serum concentrations and hypoglycemic effect of glibenclamide using solvent deposition technique. *J Pharm Pharmaceut Sci* 2005; 8(2):175-181.

26.Blume H *et al*. Pharmaceutical quality of glibenclamide products: a multinational postmarketing comparative study. *Drug Dev Ind Pharm* 1993; 19:2713–41.

27. Balan G *et al*. *In-vitro* and *in-vivo* correlation models for glibenclamide after administration of metformin/glibenclamide tablets to healthy human volunteers. *J Pharm Pharmacol* 2000; 52: 831-838.

28.Deshpande AV *et al*. Formulation and *in vitro* dissolution rate studies of glibenclamide solid dispersions. *Pharmacy World Journal* 1990; 46: 5153.

29Ghosh LK *et al*. Development and evaluation of an ideal formulation of glibenclamide by solid dispersion techniques. *Boll Chim Farm* 1998;137: 26-29.

30Kumar R *et al*. Formulation, characterization, and in vitro release of glyburide from proliposomal beads. *Drug Delivery* 2001;8: 25-27.

31.Lobenberg R *et al*. Dissolution testing as a prognostic tool for oral drug absorption: dissolution behavior of glibenclamide. *Pharm Res* 2000;17: 439-444.

32.Tashtoush BM *et al*. *In-vitro* and *in-vivo* evaluation of glibenclamide in solid dispersion systems. *Drug Dev Ind Pharm* 2004;30: 601- 607.

33.Valleri M et al. Development and evaluation of glyburide fast dissolving tablets using solid dispersion technique. *Drug Dev Ind Pharm* 2004; 30: 525-534.

34.Varma MM *et al*. *In-vitro* and *in-vivo* evaluation of fast release solid dispersions of glibenclamide. *Indian Drugs* 1992;29: 608-611.

35.Nokhodchi A *et al.* An Investigation on the Solid Dispersions of Chlordiazepoxide. *Int J Biomed Sci* 2007; 3(3):210-16.

36.M. Tabbakhian, F. Hasanzadeh, N. Tavakoli and Z. Jamshidian. Dissolution enhancement of glibenclamide by solid dispersion: solvent evaporation versus a supercritical fluid-based solvent–antisolvent Technique. Research in Pharmaceutical Sciences, 2014; 9(5): 337-350

37.Mundal SS, Pancholi SS. Formulation of glibenclamide solid dispersions by solvent evaporation technique.Journal of Chemical and Pharmaceutical Research 2012; 4(1):353-359.

38.Sigh SK *et at*. Glibenclamide loaded self-nanoemulsifying drug delivery system: development and characterization. Drug Development and Industrial Pharmacy 2010; 36(8): 933-945.

39.Yin LF *et al*. *In-vitro* and *in-vivo* studies on a novel solid dispersion of repaglinide using polyvinylpyrrolidone as the carrier. *Drug Dev Ind Pharm* 2012; 38: 1371-1380.

40.Pater JR *et al*. Preparation and structural characterization of amorphous spray-dried dispersions of tenoxicam with enhanced dissolution. *J Pharm Sci* 2012; 101: 641-663.

41.Pahovnik D *et al*. Determination of the interaction between glimepiride and hyperbranched polymers in solid dispersions. *J Pharm Sci* 2011; 100: 4700-4709.

42.Leuner C, Dressman J. Improving drug solubility for oral delivery using solid dispersions. Eur *J Pharm Biopharm* 2000; 50: 47-60.